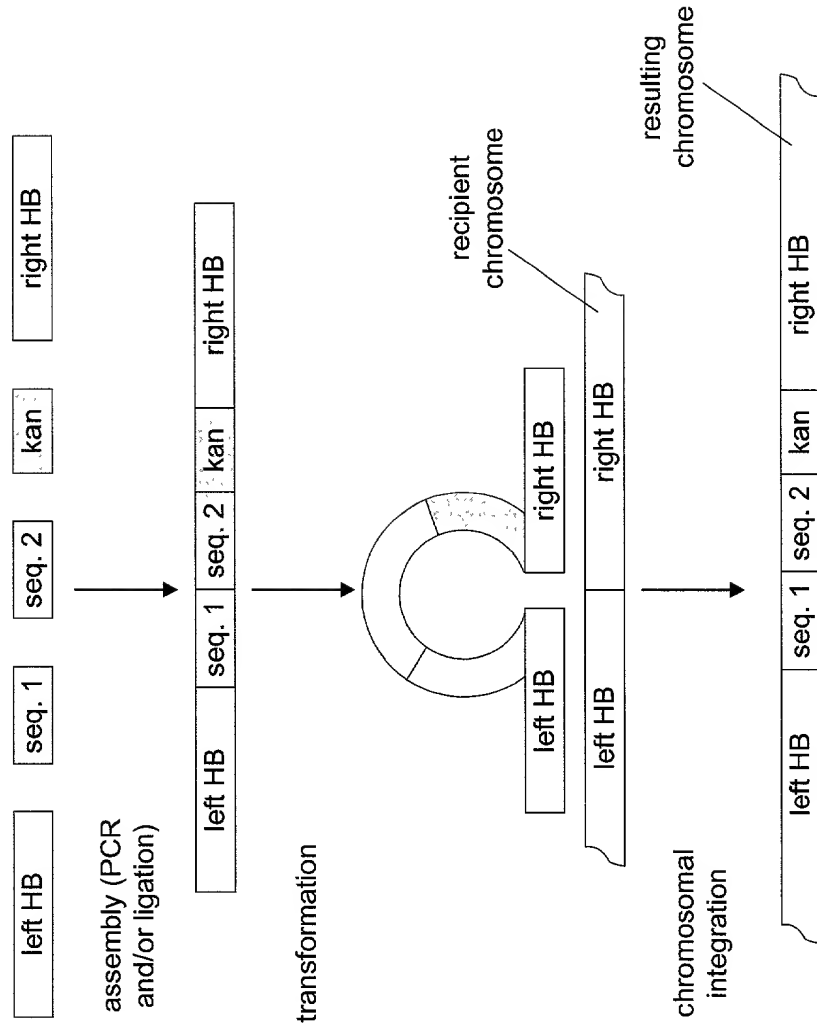
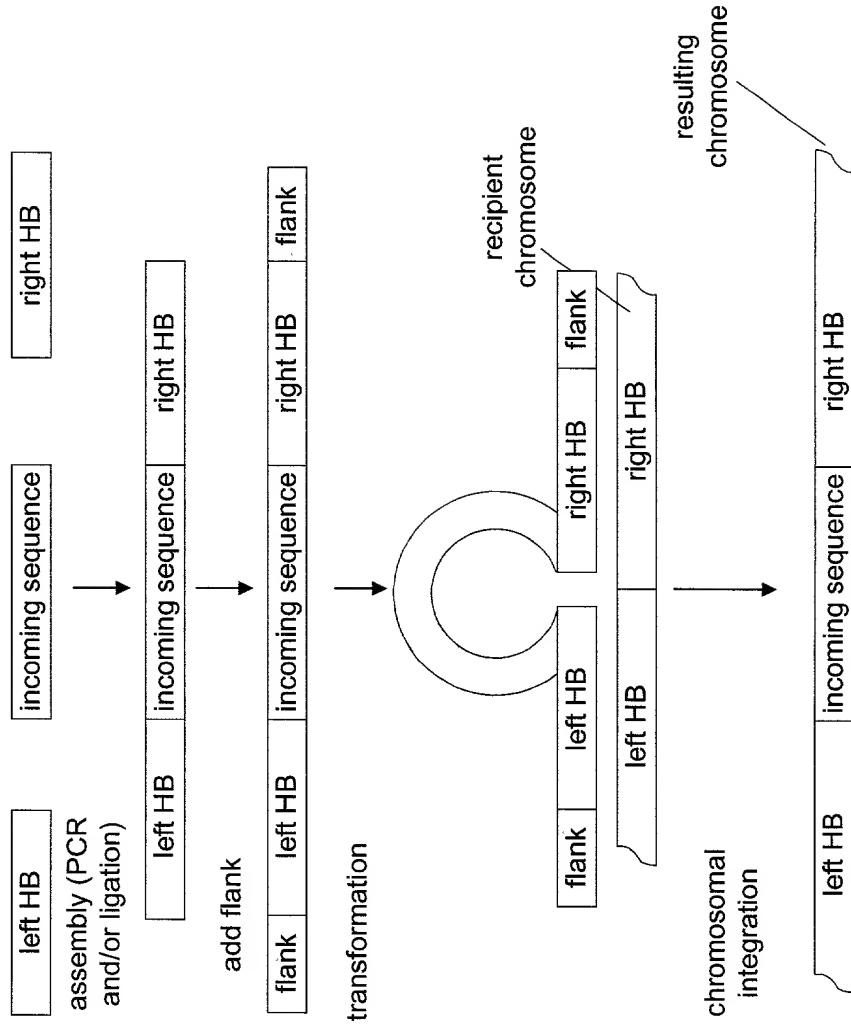


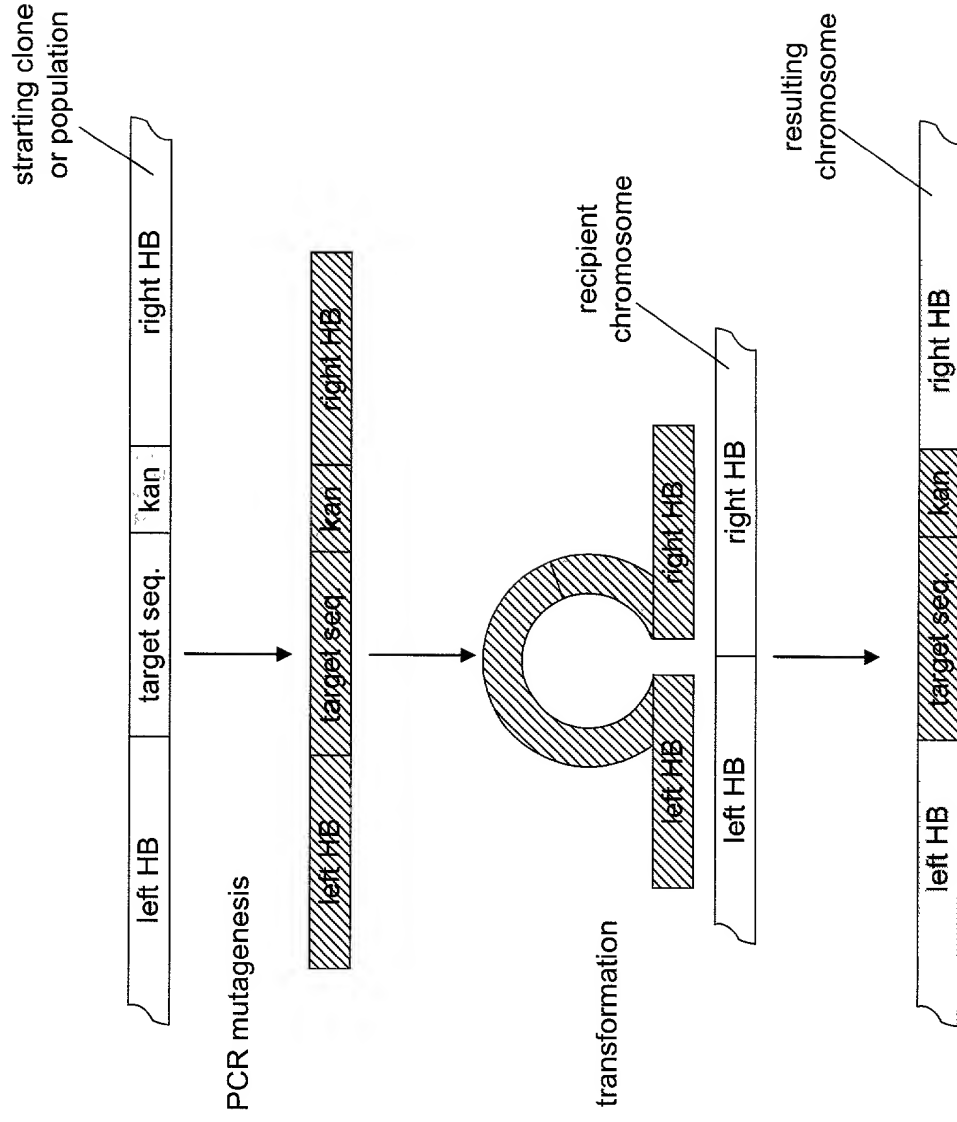
**Figure 1: Cloning by *in vitro* assembly and transformation of competent *Bacillus***



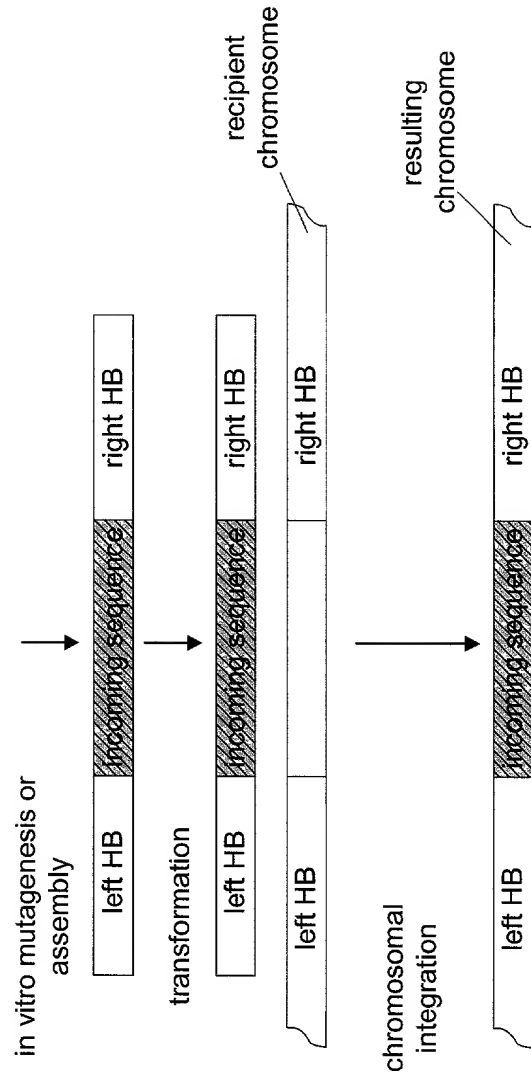
**Figure 2: Adding non-homologous flanks increases the transformation efficiency.**



# Figure 3: PCR mutagenesis of a region of the Bacillus chromosome



**Figure 4: Maximizing the homology between the transforming DNA and the target region of the chromosome increases the transformation efficiency.**



**Figure 5: Using a competent host that carries an inactive version of the marker gene, used to select transformants**

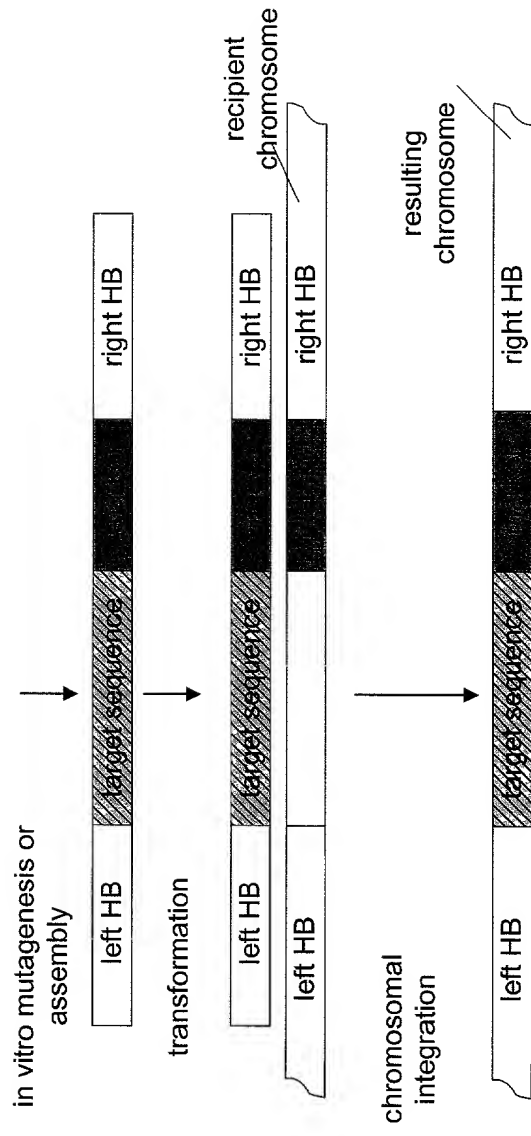
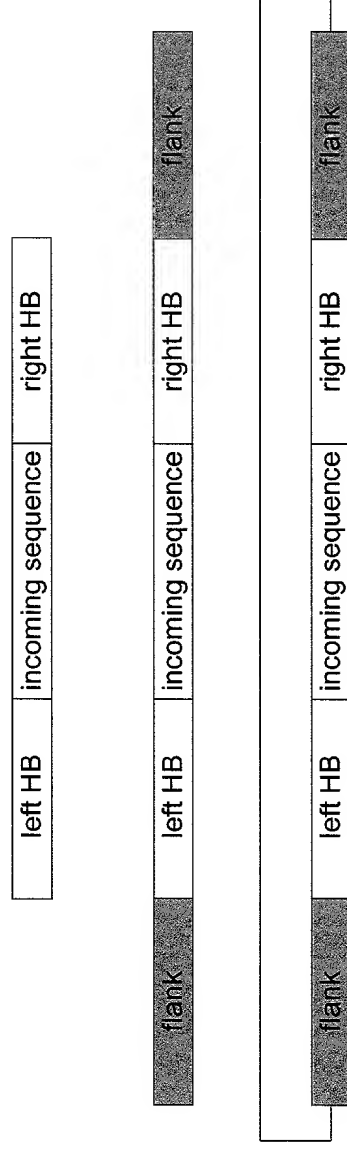
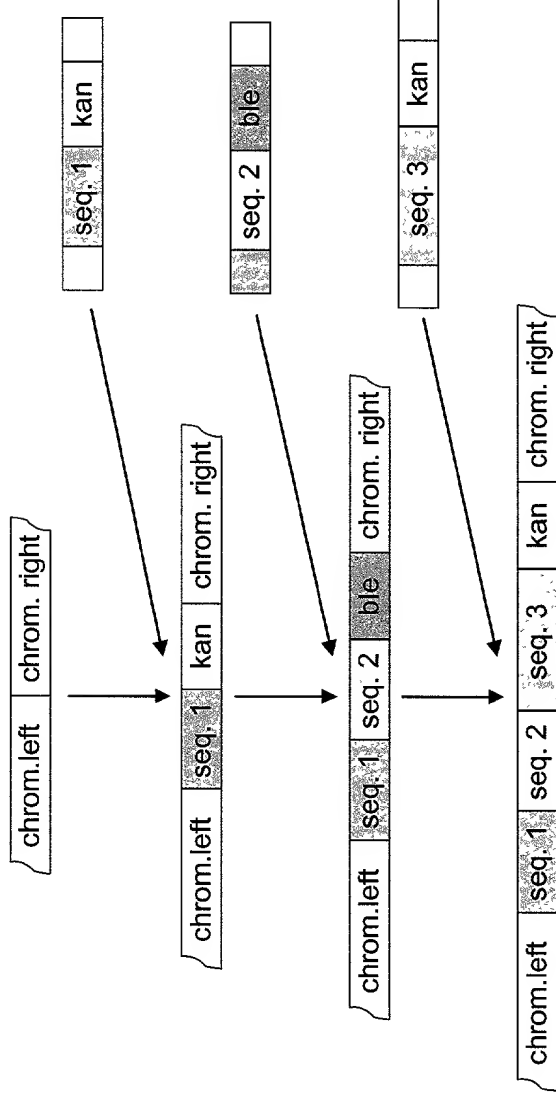


Figure 6: *General structure of transforming DNA*



## Figure 7: *Bacillus* strain construction by iterative marker replacement



### Advantages

- Replacement allows one to verify that construct went into target locus.
- Large sequences can be assembled in vivo.
- Avoids repeat PCR amplification
- Entire construct can be moved by transduction or transformation.
- Two markers are sufficient to introduce an unlimited number of genes.

Figure 8A

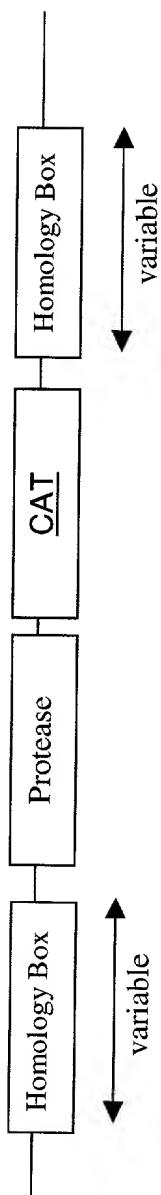
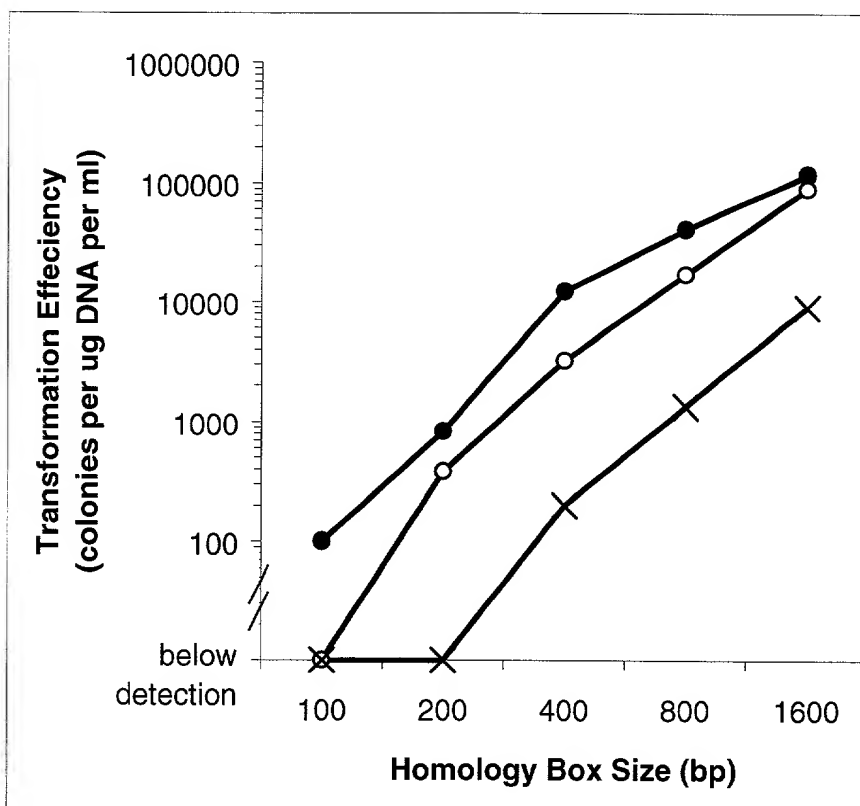




Figure 8B. Optimization of homology box size



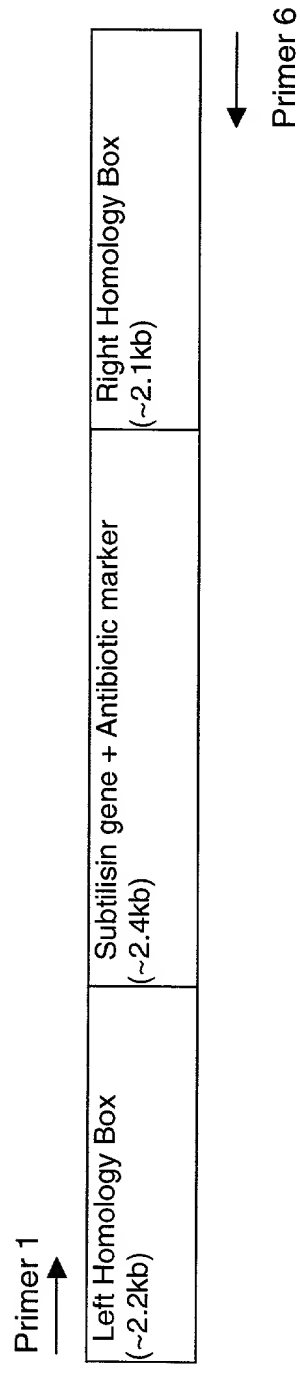


Figure 9

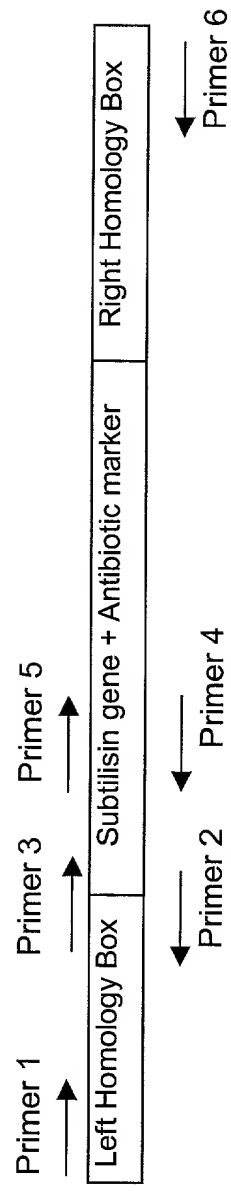


Figure 10

Figure 11

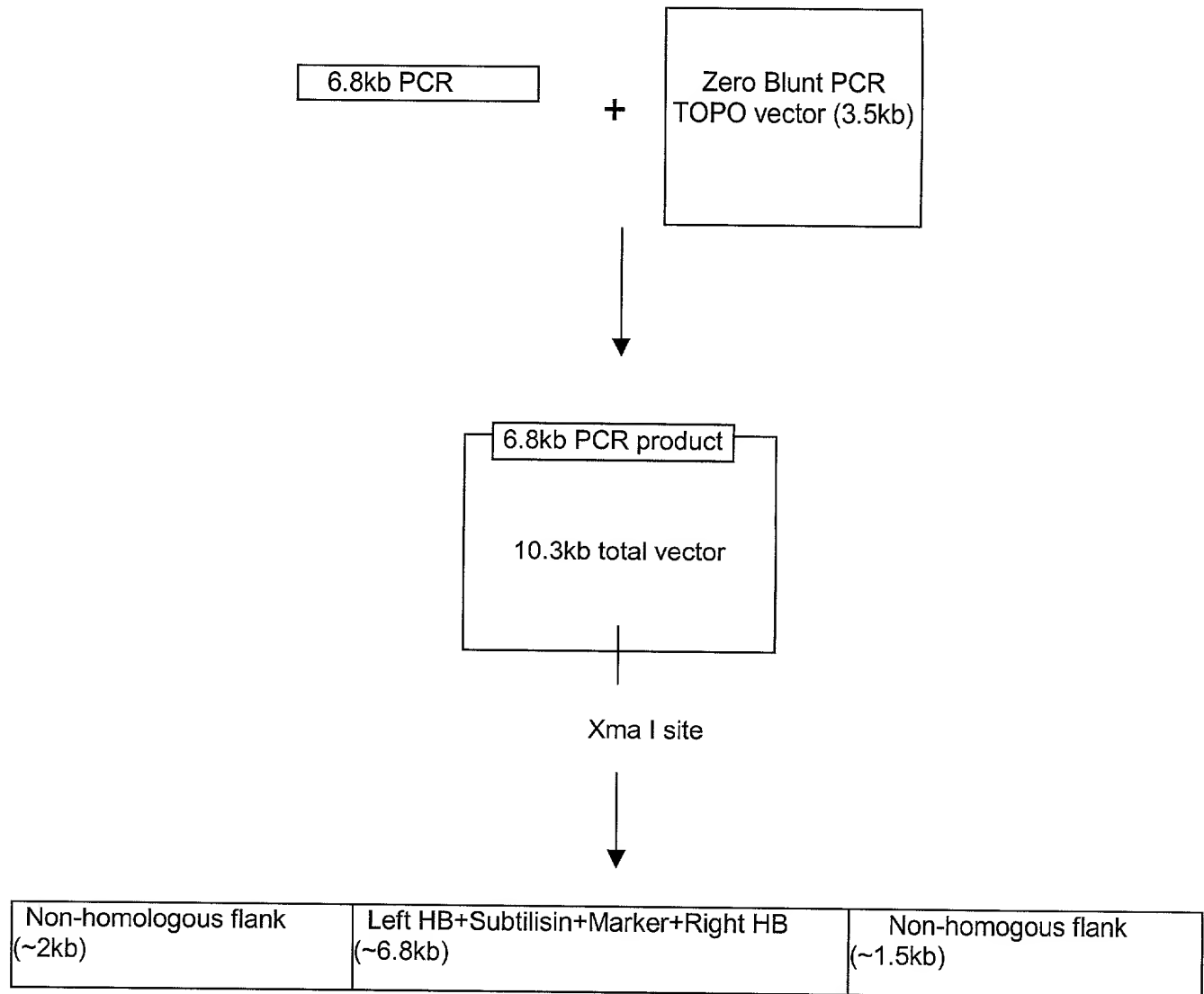


Figure 12

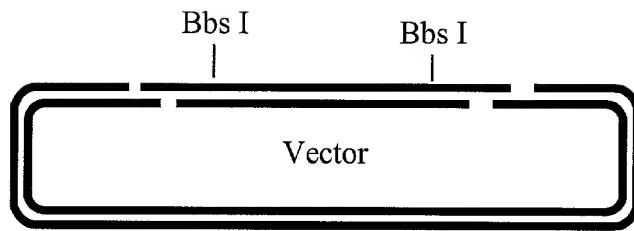
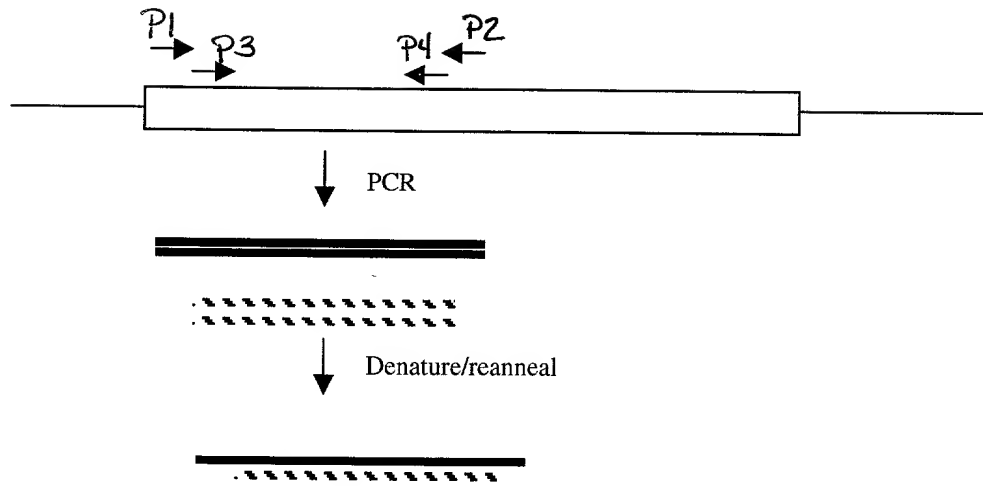
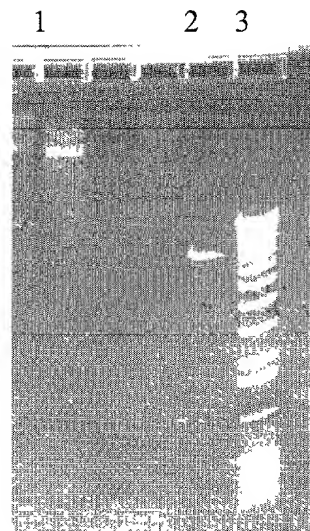


Figure 13



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Figure 14



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